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Purification efficiencies and microbial community structure of integrated vertical-flow constructed wetland for domestic wastewater treatment during acclimation period

Wu Su-qing[a,b], Chang Jun-jun[a,b], Dai Yanran[a,b], Wu Zhen-bin[a], Liang Wei[a,*]

[a] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*
Tel. +86 27-68780045; Fax +86 27-68780675; email: liangwei02@tsinghua.org.cn
[b] *Graduate University of the Chinese Academy of Sciences, Beijing 100049, China*

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ABSTRACT

Two parallel pilot-scale integrated vertical-flow constructed wetland systems (IVCWs) were employed to study the removal efficiencies for domestic wastewater and changes of microbial community structure during acclimation period. The results indicated that the acclimation period for common pollutants removal was 40 days. The mean removal rates during acclimation period were achieved for COD (77.02%), TN (57.21%), NH_4^+-N (45.63%) and TP (67.78%), respectively. Fatty acid methyl esters (FAME) analysis and function groups PCA analysis demonstrated the microorganism community structure during the acclimation period realized stable after 90 days' operation. Based on the results of purification effects and microbial community structure, 90 days was probably optimal acclimatization period for IVCW system under this experimental condition.

Keywords: Integrated vertical-flow constructed wetland; Acclimatization period; Domestic wastewater; Purification efficiencies; Microbial community structure; Fatty acid methyl ether

1. Introduction

Constructed wetland is an ecological technique involving an interactive combination of physical, chemical and biological processes to achieve wastewater purification [1–3]. Owing to its favorable landscape, higher purification efficiency and other multiple benefits [4], constructed wetland has been applied to treat a variety of wastewaters, such as storm runoff and non-point source pollution [5,6], domestic wastewater especially in rural communities [7,8], agricultural drainage, secondary wastewater effluent [9], and so on. Among them, domestic wastewater has been studied intensively. Moortel et al. [10] studied surface flow (SFCWs) and subsurface flow (SSFCWs) to treat combined sewer overflows, the average removal rates were achieved for COD (60.8 ± 7.1%), TN (36.6 ± 3.3%) and TP (36.0 ± 5.0%) in the SFCWs, and COD (88.1 ± 3.5%), TN (96.7 ± 1.9%) and TP (71.1 ± 7.7%) in the SSFCWs. Saeed and Sun [11] studied the combination of three wetland system with traditional and alternative substrates for removing contaminant from synthetic domestic wastewater, the average COD, TN, NH_4^+-N and TP removal efficiencies were over 65.0%, 72.3%, 99.4% and 94.0%, respectively.

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*Corresponding author.

Wang et al. [12] employed a novel three-stage vermifiltration (VF) system to enhance the rural domestic sewage treatment performance, the average COD, TN and TP removal efficiency were 83.1%, 60.2% and 98.4%, respectively.

Any ecological system needs a period of time to adapt environmental changes [13,14]. Therefore, before a new constructed wetland system is formally put into operation, an acclimation period is required to allow the system to perform properly [15–17]. Nevertheless, most references have been focused on the constructed wetland's mature operation period; systematic studies on purification effects during acclimation period were rarely reported [18].

Phospholipid fatty acids (PLFAs) are important components of microorganism cell membrane and they can reflect the microorganism community structure in the treatment system [19]. FAME analysis is a method for the direct extraction of fatty acids from soil or sediment microorganisms [20], it allows for a rapid and quantitative determination of microbial community profiles, handling a large sample size, as well as overall screening in a pilot field study [21]. It has been widely applied in bioremediation studies to estimate microbial viable biomass, community structure, nutrient status and physiological stress responses [22,23]. In recent year, FAME analysis has been successfully used to analyze microorganism community structure in the constructed wetland system [24].

Integrated vertical-flow constructed wetland (IVCW), as a new and efficient wetland technology, has been applied widely in China [8]. Using new pilot-scale IVCW systems, this study was conducted to evaluate the variation of purification efficiencies and microbial community structure changes during acclimation period, in order to establish and provide scientific basis for better understanding of application and operation management of the constructed wetland for treating domestic wastewater.

2. Materials and methods

2.1. Experimental system

Two parallel pilot-scale IVCW, each with a down-flow chamber (1 m × 1 m × 1 m) in series with a up-flow chamber (1 m × 1 m × 1 m) (Fig. 1), were constructed in Wuhan, China. Gravel of 10–20 mm diameter in size was filled to a depth of 50 cm and 40 cm for the down-flow chamber and up-flow chamber, respectively, followed by a 35 cm thick layer of 2–10 mm diameter gravel. The porosity of the substrate was estimated to be 0.40 and the effective volume of the wetland bed was 0.6 m^3.

Two species of macrophytes, *Arundo donax* and *Canna indica* were planted in the down-flow and up-flow chamber at a density of 6 plants/m^2, respectively.

2.2. Operation conditions

The influent was induced intermittently twice a day at a loading rate of 0.25 $m^3\ d^{-1}$, yielding a hydraulic loading rate of 125 mm/d, and the theoretical hydraulic retention time (HRT) was 2.4 d. The study was carried out from April 23 to August 23, 2011 with an average ambient temperature of 27.78 °C. In order to minimize variability in the experiment, simulated domestic wastewater was used in the experiment. The influent characteristics were summarized in Table 1.

2.2. Sample collected

Water samples were collected from the influent and effluent regularly, and the sampling frequency was scheduled for once every two days during the first 20 days, once

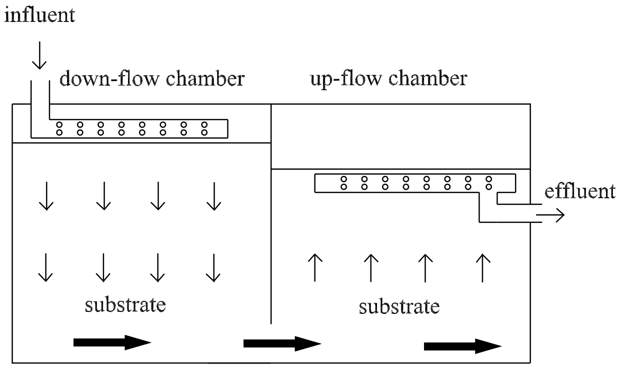

Fig. 1. A schematic diagram of IVCW.

Table 1
Characteristics of the influent

Parameters	Mean value ± SD
COD (mg/L)	104.35 ± 20.43
TN (mg/L)	12.12 ± 1.42
NH_4^+-N (mg/L)	9.11 ± 1.36
NO_3^--N (mg/L)	1.71 ± 0.77
NO_2^--N (mg/L)	0.16 ± 0.13
TP (mg/L)	0.94 ± 0.14
Conductivity (us/cm)	413.17 ± 32.63
pH	7.43 ± 0.56
DO (mg/L)	3.94 ± 1.62
T (Ambient, °C)	27.78 ± 8.23

Note: n = 60 for pH, DO and conductivity; n = 27 for the pollutant concentrations.

every four days during the middle 30 days, once a week during the last 70 days.

According to Ref. [24], the substrate samples were collected from five representative sites of the IVCW system and combined in a composite sample. All litter was removed and the samples were taken to the laboratory in sealed polypropylene bags and stored at 4 °C.

2.3. Water quality analysis

pH, electric conductivity (EC), dissolved oxygen (DO), and temperature were determined by Orion 5-star portable pH/conductivity/DO multimeter (Thermo Fisher Scientific Company, USA). COD (DRB 200, Hach, USA), TN, ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N) and TP were measured according to the standard methods [25].

Pollutant removal efficiency was calculated by the percentage of deduction in concentration for each pollutant as follows: removal efficiency = $(1 - C_{eff}/C_{inf}) \times 100\%$, where C_{inf} and C_{eff} are the influent and effluent concentrations in mg/L.

2.4. Fatty acid methyl esters analysis

Fatty acid methyl esters (FAME) analysis of substrate samples employed a mild alkaline methanolysis method [20]. Fifteen milliliter of 0.2 M potassium hydroxide (KOH) in methanol was added into a 35-ml centrifuge tube containing 3 g of freeze–dried substrate. The contents of the tubes were mixed and incubated at 37 °C for 1 h, during which ester-linked fatty acids were released and methylated. The tubes were vortexed every 10 min during the incubation period. Then, 3 ml of 1.0 M acetic acid was added to neutralize the pH of the tube's contents. EL-FAMEs were partitioned into an organic phase by adding 10 ml hexane followed by centrifugation at 4500 rpm for 15 min. After the hexane layer was transferred into a clean glass tube, the hexane was evaporated under N_2 steam. In the final step, FAMEs were dissolved in three aliquots of 200 µl of hexane:methyl*tert* butyl ether (1:1) and transferred to an amber vial for gas chromatography (GC) analysis. The MIDI peak identification software (MIDI, Inc., Newark, DE) was used to identify individual fatty acids. Fatty acid peaks were identified using 26-component bacterial acid methyl ester (BAME) Mix (Supelco, USA).

Fatty acids (FAs) are designated by the total number of carbon atoms, followed by a colon and the number of double bonds. Then a "ω" and a number show the position of the initial double bond from the methyl end of the chain, sometimes followed by a "c" or "t" for *cis* or *trans* configuration, respectively. The prefixes "i" and "a" refer to methyl branching at the iso- and anteiso-positions, respectively. Cyclopropane FAs have the prefix "cy".

2.5. Statistical analysis

The experiments were conducted in triplicate, and data were summarized and reported as mean values ± standard deviation (SD). Comparison of the averages was carried out by Independent-Samples T-test and ANOVA test post-hoc Tukeys, using the software of SPSS 16.0 (SPSS Inc., Chicago, IL, USA), significant differences was set at $p < 0.05$. Principal component analysis (PCA) was performed to explore the variability of the microbial EL-FAME composition.

3. Results

3.1. Removal rate of COD in IVCW

The removal efficiencies for COD during acclimation period and the Independent-Samples T-test of COD removal efficiency are shown in Fig. 2 and Table 2, respectively.

As seen in Fig. 2, during the first 20 days (April 23 to May 13), the COD removal efficiency fluctuated sharply in the early 10 days, and then became stable. Significant difference of COD removal efficiency was found between 0–10th days and 10–20th days (Table 2). So the acclimation period for COD removal was 20 days, and the mean removal efficiency was 77.02 ± 17.01%. After the acclimation period, the mean removal efficiency was 83.35 ± 6.82% and the effluent COD concentrations were between 6.25 and 28.0 mg/L, which can meet the national standards of surface water environment quality V in China (GB3838-2002).

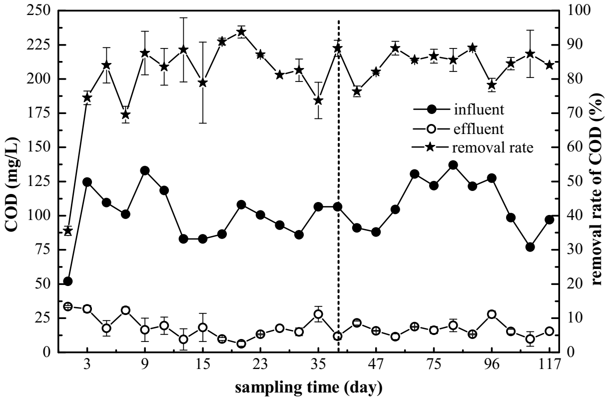

Fig. 2. The removal efficiency for COD.

Table 2
Independent-Samples T-test of COD and TN removal efficiencies

Time (days)	Independent-Samples T-test	
	COD	TN
0–10th vs 10–20th	$t = -2.521$, df = 18, $p = 0.021^*$	$t = 2.735$, df = 18, $p = 0.029^*$
10–20th vs 20–40th	$t = 1.425$, df = 18, $p = 0.171$	$t = -2.536$, df = 18, $p = 0.021^*$
20–40th vs 40–60th	$t = -0.071$, df = 15, $p = 0.941$	$t = 1.185$, df = 15, $p = 0.254$

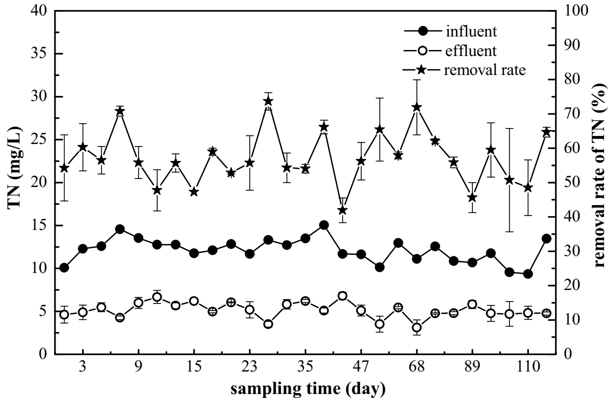

Fig. 3. The removal efficiency for TN.

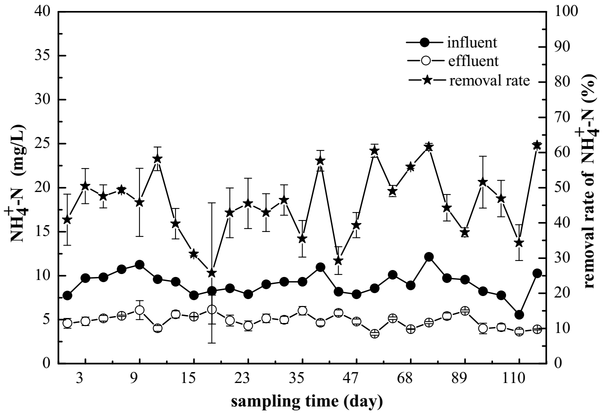

Fig. 4. The removal efficiency for NH_4^+-N.

3.2. Removal rate of N in IVCW

The removal efficiencies for N during acclimation period and the Independent-Samples T-test of TN removal efficiency are shown in Figs. 3–6 and Table 2.

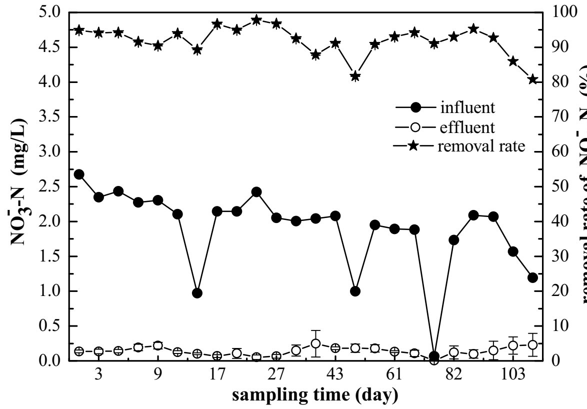

Fig. 5. The removal efficiency for NO_3^--N.

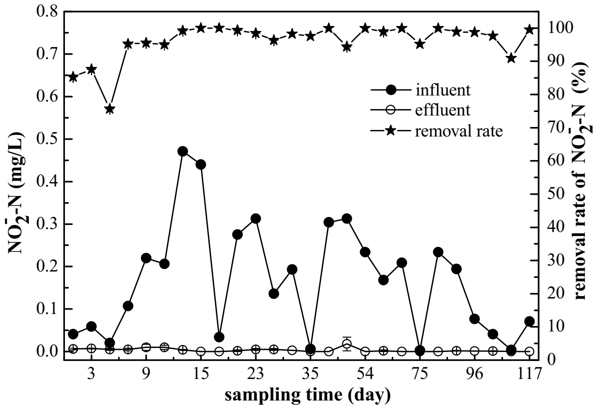

Fig. 6. The removal efficiency for NO_2^--N

As shown in Figs. 3–6, during the whole experimental period, the TN removal efficiencies fluctuated during the first 40 days, and a mean removal rate of 57.21 ± 7.38% was achieved.

As for NH_4^+-N, during the operation period, the NH_4^+-N removal efficiency was 45.63 ± 9.86%, and the average effluent NH_4^+-N concentration was 4.99 mg/L.

As for NO_3^--N and NO_2^--N, the system kept as high as above 90% removal efficiencies, and the average effluent NO_3^--N and NO_2^--N concentrations were 0.15 mg/L and 0.005 mg/L, respectively.

Significant difference of TN removal efficiency was observed among 0–10th, 10–20th and 20–40th days (Table 2). Therefore, the acclimation period for N removal was 40 days.

3.3. Removal rate of TP in IVCW

The removal efficiencies for TP during acclimation period are shown in Fig. 7.

As shown in Fig. 7, during the experimental period, the TP removal efficiency showed a slight fluctuation at the first 20 days, but no significant difference was found.

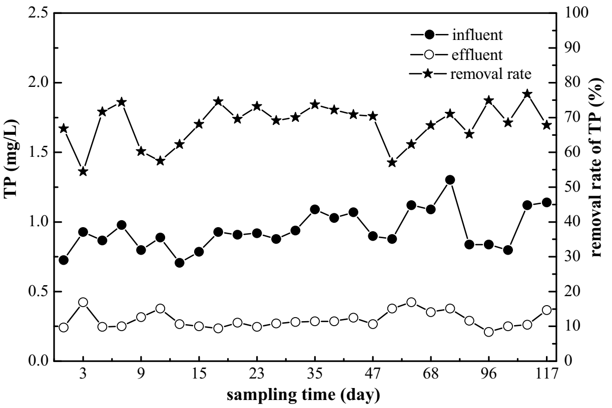

Fig. 7. The removal efficiency for TP.

The mean TP removal rate was 67.78%, and the effluent TP concentrations were between 0.21 and 0.42 mg/L, which meet the national standards of surface water environment quality V in China (GB3838-2002).

3.4. Microbial EL-FAME profiles

A total of 26 kinds of fatty acids were identified to characterize the microbial community structure in all substrate samples. The kinds and relative contents of FAMEs of sample were determined (Table 3), 13 kinds of FAs was found from the samples.

As seen in Table 3, saturated FAs dominated the EL-FAME profiles by 35.66–52.00%. Second was branched FAs (19.19–39.31%), followed by the monounsaturated FAs (MUFAs) (15.67–30.07%). Cyclopropane FAs were present with relatively low occurrence (7.96–8.20%).

The distribution of FA groups in substrate samples from IVCW under different operation time was summarized in Fig. 8.

As shown in Fig. 8, the relatively abundance of saturated FAs and branched FAs increased with the operation time, which reached the maximum value on the 30th and 60th day, respectively, then decreased gradually. Meanwhile, MUFAs and cyclopropane FAs decreased on the 30th and 60th day, and then increased gradually. The relatively abundance of all kinds of FAs groups realized stable on the 90th day, and no significant difference ($p > 0.05$) between 90th and 120th day was found.

The PCA analysis for change of microorganism community structure during the experiment was summarized in Fig. 9. The data matrix used in the PCA analysis

Table 3
EL-FAME composition (% of total FAs) in the substrate sample

FAs	day 0	day 30	day 60	day 90	day 120
Saturated					
14:0	5.71 (0.31)	ND	ND	5.47 (0.13)	5.29 (0.02)
15:0	6.05 (0.12)	ND	ND	ND	ND
17:0	8.19 (0.03)	13.36 (0.06)	ND	8.06 (0.09)	7.71 (0.06)
16:0	12.49 (2.11)	19.09 (3.31)	20.56 (1.58)	13.34 (2.17)	12.57 (1.02)
18:0	11.79 (1.49)	19.56 (2.07)	21.38 (0.61)	10.79 (1.14)	10.08 (0.63)
Branched					
i15:0	5.67 (0.11)	9.56 (0.06)	11.73 (0.05)	5.83 (0.19)	6.04 (0.08)
a15:0	5.57 (0.15)	8.70 (0.21)	10.44 (0.20)	5.38 (0.24)	5.88 (0.05)
i16:0	ND	ND	ND	5.96 (0.06)	6.25 (0.04)
i17:0	7.95 (0.01)	14.06 (0.02)	17.14 (0.05)	8.38 (0.04)	8.15 (0.01)
Monounsaturated fatty acids (MUFA)					
16:1–9	11.02 (0.07)	15.67 (1.08)	18.75 (0.28)	9.61 (0.30)	10.05 (0.08)
18:1–9c	9.72 (0.01)	ND	ND	9.71 (0.05)	10.37 (0.22)
18:1–9t	7.80 (2.13)	ND	ND	9.27 (0.19)	9.65 (0.13)
Cyclopropane					
cy17:0	8.05 (0.03)	ND	ND	8.20 (0.08)	7.96 (0.01)

ND: not detectable; mean and standard deviation values of triplicates in bracket are shown.

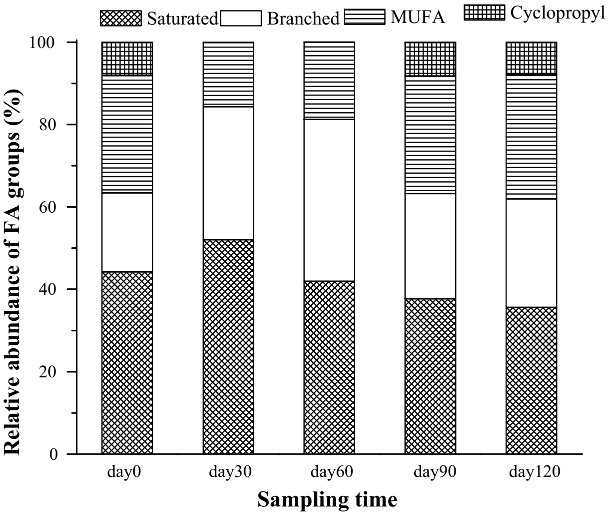

Fig. 8. The distribution of FA groups under different operation time.

was composed of the relative abundances of six fatty acid groups and three special fatty acid ratios from 30 substrate samples. Principal components (PCs) 1 and 2 explained 58.07% and 23.92% of the total variations, respectively. The PCA revealed that significant variance for the FAME function groups in the constructed wetland substrate were observed from the 0th to 60th days, while no obvious variance was found between 90th and 120th day, which also indicated substrate microbial community structure had realized stable after 90 operation days.

4. Discussions and conclusion

As we all know, the purification of constructed wetland for COD and nitrogen involves wetland plants and microbes. For a new constructed wetland to function properly, an acclimation period is needed for both wetland plants and microbes to grow to a certain stage. In this study, the acclimation period for purification efficiencies under our experiment condition was 40 days. During the acclimation period, the mean COD removal rates was 77.02%, which was higher than Fountoulakis et al. (60.01%) [26], but lower than Zhao et al. (88.19 ± 4.81%) [27]. The removal efficiency of TN (57.21%) was similar to Wang et al. [12] and Wu et al. [8]. Meanwhile, removal rates of NH_4^+-N (45.63%) was much lower than Wu et al. [8] and Xiong et al. [9]. As for TP, the mean removal rate (67.78%) was similar to Wu et al. [8] and Chung et al. [28], but lower than Xiong et al. [9], Dong et al. [29] and Wang et al. [12].

Balcombe et al. [30] and Spieles [31] reported that mitigation wetlands may take years or even decades to mature. Weaver et al. [32] also indicated that very low variance in the FAMEs within the constructed wetland after 1 year operation, and reached a greater temporal stability. Atlas et al. [33] proved that a reduction in microbial diversity generally occurred after a nutrient amendment. In this study, a large proportion of variations in microbial community profiles were associated with sampling time, which was assumed to represent the acclimation process in the IVCW. A significant shift in microbial community structure among different sampling times (Fig. 8 and 9) was found, FAME analysis

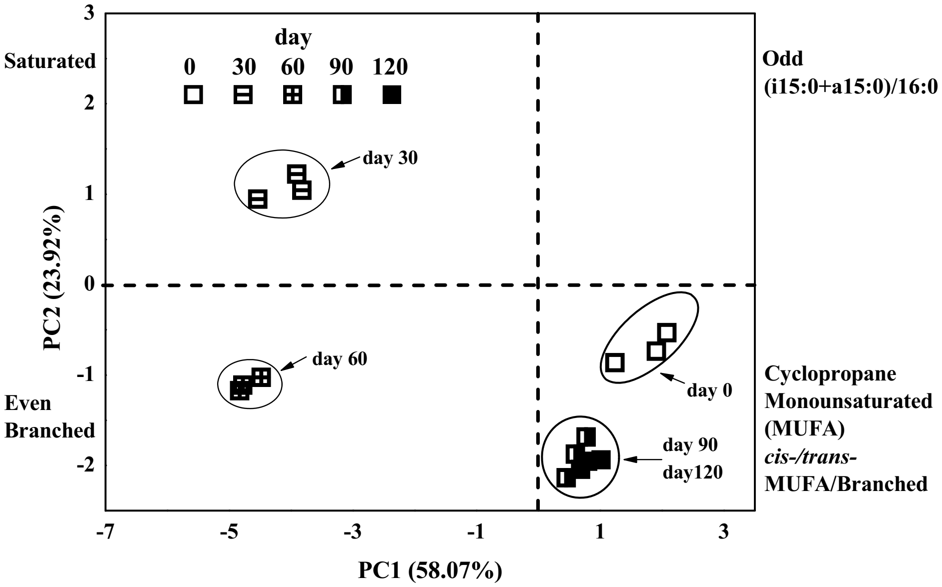

Fig. 9. PCA analysis of microbial EL-FAME groups with sampling time.

demonstrated a significant change have taken place during the 90 days' operation, and then kept stable throughout the experimental period. The acclimation period for microbial community structure under our experiment condition was probably 90 days.

The acclimation period for constructed wetland can be affected by many factors. Baclombe et al. [30] concluded that the time to maturity would be affected by the initial conditions. Therefore, the extent of purification and the length of this period vary depending on water quality, wastewater compositions, pH, temperature, hydraulic loading rate, hydraulic retention time and other relevant factors. In our another study, about 60 days was needed to realize stable removal for common pollutants, when the initial average concentration of COD, TN, NH_4^+-N, TP were 280.67 mg/L, 30.88 mg/L, 20.67 mg/L and 3.21 mg/L, respectively.

In general, purification effects and microbial community structure of integrated vertical-flow constructed wetland during the operation period have experienced a period of fluctuation and stable process. Based on the results of purification efficiencies and microbial community structure, 90 days was probably optimal acclimatization period for IVCW system under this experimental condition.

Acknowledgements

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